

Scheme 2. Illustration of hierarchical self-assembly process. Nanoribbons are formed through assembly of chimeric oligomers (**Py-a**) in a first step. Subsequent hybridization of oligonucleotide **1b** to the DNA part of the nanoribbons leads to formation of duplexes containing GC base pairs at their ends. Networks are then formed *via* cooperative blunt end stacking interactions.

DNA-grafted SPs are typically performed by slow annealing. Thus, a 2 μM solution of **Py-a** in aqueous buffer (10 mM sodium phosphate, pH=7.0 and 250 mM sodium chloride) is cooled from 95° to 20°C using a gradient of 0.1°C/min. Stacking interactions between pyrenes drive the self-assembly of polymeric ribbons. The polymerization process leads to the development of two distinct absorption bands in the UV/vis spectrum at 335 (H-band; S₀→S₁ transition) and 305 nm (J-band; S₀→S₂ transition; see Figure 1A).^{35,36} The assembly/disassembly process is most conveniently followed by changes of the 305 nm band (Figures 1B-C; for monitoring at 260 nm see SI, Fig. S11). Figure 1B shows the assembly of ribbons from **Py-a** upon cooling. The polymerization occurs *via* a single transition that starts at approx. 85 °C. The process is reversible, albeit some hysteresis is observed. Surprisingly, if the same procedure is performed in the presence of the complementary oligonucleotide **1b**, a second transition appears below 30°C (Figure 1C). The change in the intensity of the J-band reflects a conformational reorganization of the supramolecular pyrene backbone. The sharpness of this transition, characterized by the full-width at half-maximum (fwhm)²⁵ of 1.5°C (melting) and 2.5°C (annealing), suggests a high degree of cooperativity. Also this process is reversible and shows hysteresis. In contrast, annealing/melting curves for a system containing **Py-a** and the non-complementary oligonucleotide **1a** exhibit only a single transition below 85°C, which coincides with the formation of **Py-a** nanoribbons (Figure 1D).

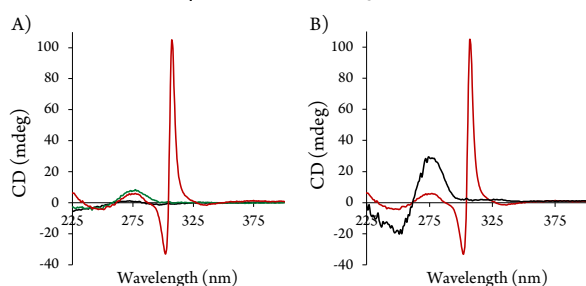


Figure 2. CD spectra at 20°C; A) **Py-a** (black), **Py-a + 1a** (green), **Py-a*1b** (red) prepared by slow annealing (conditions: as in Figure 1); B) **Py-a*1b** before (red) and after (black) addition of **1a** (conditions: as in Figure 1, except **1a** was used at 12 μM conc.).

Circular dichroism (CD) spectroscopy provides further insight in the nature of the transition observed around 30°C. While the CD spectrum of the **Py-a*1b** system resembles the one of B-DNA below 300 nm, it exhibits a strong exciton-coupled signal (Figure 2A, red curve) in the 300-320 nm region at 20°C. The bisignate signal (+307/-303 nm) corresponds to the J-band of assembled pyrenes.^{37a} Heating to

35°C leads to the disappearance of the signal (see SI, Figure S2), which clearly shows that it is linked to the transition taking place in this temperature range. The appearance of the exciton coupled signal represents a change in the relative orientation of the transition dipole moments.^{37b} This suggests that the stacking arrangement of the pyrenes and, hence, their electronic interaction is altered during the observed process. In contrast, the samples prepared either from **Py-a** alone or **Py-a + 1a** are CD silent in the 300-320 nm region (Figure 2A, black and green curves). Thus, the low temperature transition only occurs when **1b** (*connector strand*) is hybridized to the DNA part of **Py-a**.

Atomic force microscopy (AFM) was used to correlate the spectroscopic data with the morphological appearance of aggregates. Supramolecular polymers were deposited and visualized on amino-modified mica surface. Self-assembly of **Py-a** leads to the formation of ribbons that exhibit a length of several hundred nanometers and are randomly distributed on the surface (Figure 3A). The **Py-a + 1a** mixture leads to identical results (Figure 3B). In the complementary **Py-a*1b** system, however, polymers exist as high-density networks (Figure 3C), reminiscent of haystacks in a field, rather than as individual ribbons. The formation of networks is confirmed by transmission electron microscopy (TEM; Figure S10).

The two transitions appearing in the annealing curves of **Py-a** in the presence of complementary oligonucleotide **1b** reflect the hierarchical structural organization of chimeric oligomers. Based on the combined spectroscopic and morphologic data, we propose a model for network formation as illustrated in Scheme 2. The first transition is due to the formation of nanoribbons via supramolecular polymerization of **Py-a**. The second cooperative transition occurs only in the presence of the complementary oligonucleotide **1b**. DNA hybridization between **1b** and the single-stranded oligonucleotides grafted onto the pyrene nanoribbons results in the formation of duplexes. These duplexes are arranged along the edges of the ribbons (see Scheme 2) and contain GC base pairs at their termini. Cooperative interactions between individual ribbons through coaxial stacking of these blunt-ended GC base pairs lead to network formation. We assume that each ribbon, through short patches of a few GC basepairs, is connected to neighboring ribbons; long-range collinear stacking of ribbons is unlikely. The importance of blunt end stacking interactions for the controlled assembly of DNA nanostructures and devices is well documented.^{5,6,15,38-40} Coaxial stacking⁴¹⁻⁴⁴ of GC base pairs is significantly stronger than of AT base pairs.^{45,46} This is also observed in the present case. In oligomer **1c**, the two 3'-terminal bases are switched in comparison to **1b** (Scheme 1). The annealing curve of oligomer **Py-c** in the presence of **1c** shows only a single transition occurring at 80±5 °C (Figure S7). Thus, the blunt-ended AT base pairs, which are formed by hybridiza-

tion of **1c** with **Py-c**, do not support network formation due to decreased stability of their coaxial stacking interactions. The influence of base composition on network formation was further elicited by a series of control oligonucleotides differing in length and nucleotide sequence (see SI, Scheme S1). The data show that mismatches, overhanging nucleobases or shorter duplexes have the expected negative impact on the formation and the stability of networks. Furthermore, the hierarchical assembly is not limited to the **Py-a*1b** pair. Identical results are obtained with the combination **Py-b*1a** (SI; Figure S1), which also contains GC blunt ends.

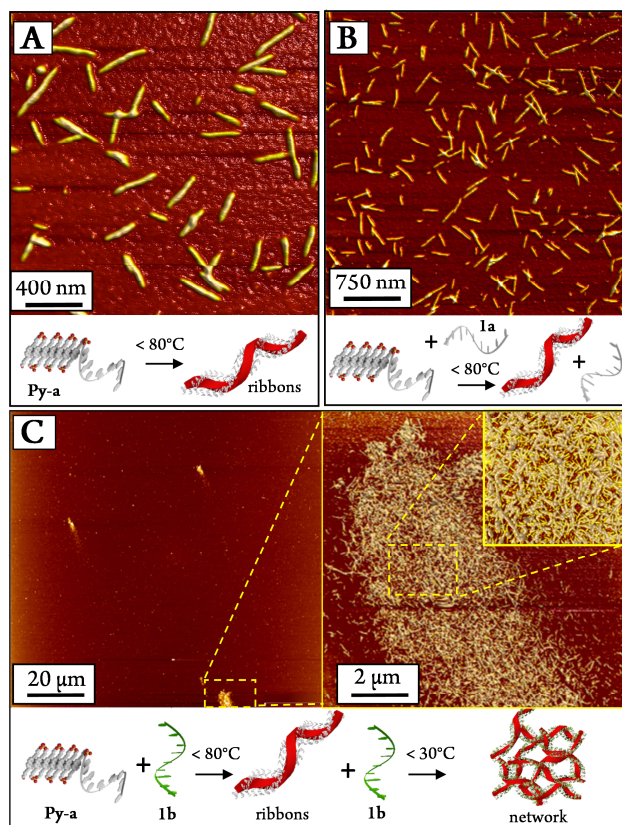


Figure 3. AFM images and illustration of supramolecular assemblies formed from **Py-a** (A), **Py-a + 1a** (B) and **Py-a*1b** (C). Conditions as in Figure 2.

The supramolecular nature of the interaction of nanoribbons allows reversing the network formation under isothermal condition, as shown by competition experiments. Thus, addition of the separator strand (**1a**, 2-fold excess over **1b**) to the **Py-a*1b** network at 20°C results in the disassembly of the aggregates (illustrated in Scheme 2). Formation of the duplex **1a*1b** ($T_m=47^\circ\text{C}$, SI Figure S5) leads to the removal of the connector strand **1b** from the network. Complete disappearance of the network is accomplished within 2 hours (SI, Figure S4C). AFM imaging shows only individual ribbons after addition of **1a** (Figure S8). The disassembly process is also confirmed by the disappearance of the CD signal centered around 305 nm (Figure 2B).

In conclusion, we have demonstrated that chimeric pyrene-DNA oligomers assemble into extended networks via hierarchical assembly pathways. The first step, self-assembly of oligomers into helical nanoribbons, is driven by aromatic stacking interactions among pyrene units. The supramolecular polymerization is independent from the nucleotide sequence of the DNA part. The second step, aggregation of individual nanoribbons into extended networks, only takes place in the

presence of a complementary oligonucleotide. Hybridization leads to the formation of duplexes along the helical nanoribbon core. Coaxial stacking interactions of blunt end GC base pairs trigger the formation of a network in a highly cooperative process. The networks can be disassembled by destroying the coaxial stacking interactions either by heating or through addition of a separator strand. Supramolecular polymeric networks of this type may be relevant for the development of DNA-based smart materials, such as stimuli-responsive carriers of active ingredients.

ASSOCIATED CONTENT

Supporting Information

Additional spectroscopic (UV-vis, CD) and microscopic (AFM and TEM) data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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